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Peritoneal Washing Cytology: Prognostic Value of Positive Findings in Patients with Gastric Carcinoma Undergoing a Potentially Curative Resection

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Background and Objectives: Free cancer cells in the abdominal cavity exfoliated from a tumor are considered to be responsible for peritoneal dissemination, the most frequent pattern of failure in gastric carcinoma patients treated with curative surgery.

Methods: A prospective survival analysis was performed with 91 gastric carcinoma patients treated by potentially curative resection. Cytology was performed for all the patients. The method of Kaplan and Meier was used to construct curves with diagnosis of peritoneal dissemination and cancer death as the end points. Multivariate analysis by Cox's proportional hazards model was performed to identify independent prognostic factors of significance.

Results: Patients with a positive cytology result were confirmed to have a greater risk for recurrence in the pattern of peritoneal carcinomatosis and hence a significantly inferior prognosis. Positive cytology was the only significant independent prognostic factor among the curatively resected patients with advanced gastric carcinoma.

Conclusions: Peritoneal lavage cytology should be employed for all advanced cancer undergoing potentially curative resection for added accuracy in the stage classification. The results should also reflect the eligibility of the patients for future clinical trials involving perioperative intraperitoneal chemotherapy.

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KEY WORDS: **peritoneal dissemination; recurrence; prognosis; stage classification**

INTRODUCTION

Peritoneal carcinomatosis is the most frequent pattern of metastasis and recurrence in patients with gastric carcinoma [1,2]. Presumably, disseminated lesions originate from free cancer cells exfoliated from the cancer-invaded serosa. In order to detect these free cells, cytology of the peritoneal washing has been performed in several Japanese institutions [3–5]. The prognostic value of positive cytology findings was recently confirmed also in the West [6], and a new stage classification for gastric carcinoma, recently published by Japanese Gastric Cancer Association, employs the result of cytologic examination (Cy categories) as one of the key prognostic factors [7].

In this study, peritoneal wash cytology was performed in 91 patients with advanced gastric carcinoma who had been treated by potentially curative surgery with lymphadenectomy. (Patients who had macroscopic evidence of

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peritoneal seeding were excluded from the study.) Patients underwent a follow-up program in the Department of Gastroenterological Surgery, Aichi Cancer Center, and the prognostic value of the cytology examination was evaluated with multivariate analysis together with other known prognostic variables.

MATERIALS AND METHODS

Patients operated on between May 1995 and April 1998 who fulfilled the following criteria were eligible and were prospectively analyzed in this study: 1) histologically confirmed gastric carcinoma with the invasion of muscularis propria (pT2) or more; 2) no macroscopic evidence of peritoneal seeding or other distant metastasis; 3) potentially curative resection with complete macroscopic and histological removal of the tumor (R0 resection); 4) no treatment by neoadjuvant chemotherapy; and 5) peritoneal lavage cytology with Papanicolaou and Giemsa staining.

Cytologic examination was performed immediately after the laparotomy, prior to manipulation of the tumor. Saline (100 ml) was introduced into the Douglas pouch and recovered after gentle stirring. Half of the lavage fluid was stored for another related study [1], and the remaining 50 ml was centrifuged. The cell layer was stained with Papanicolaou and Giemsa stains and examined and classified by experienced pathologists on the basis of the presence or absence of free cancer cells. These cytologic diagnoses were rendered intraoperative, but the surgeons performed gastrectomies without compromising the extent of lymphadenectomies in the event of positive cytology results without macroscopic evidence of distant metastasis. The resected specimens were examined by the pathologists after hematoxylin eosin staining, and the depth of cancer invasion (pT categories) and the number of metastatic lymph nodes (pN categories) were evaluated for clinical staging according to the the 5th edition of TNM classification [8]. Histopathological type was classified into differentiated and undifferentiated type according to the Japanese Classification of Gastric Carcinoma [7].

The follow-up program consisted of interim history, physical examination, hematology, and blood chemistry panels, including serum tumor markers, obtained every 3 months for the first postoperative year and every 6 months thereafter. Either abdominal ultrasonography or computer tomography was performed every 6 months. Patients who underwent proximal or distal gastrectomy underwent annual endoscopic follow-up of the gastric remnants. Peritoneal recurrence, evident on the basis of clinical symptoms, digital examination, or physical findings of bowel obstruction and ascites, was confirmed by barium swallow or barium enema studies, along with paracentesis and laparotomy performed at the discretion of the surgeon.

Of the 92 patients who were considered eligible, there was a case of early postoperative death due to a sudden *Clostridium perfringens* sepsis 17 days postoperatively after a seemingly uneventful course. This patient was excluded, and the remaining 91 patients underwent the survival analysis. Two other deaths unrelated to cancer were treated as censored cases. The median duration of follow-up was 760 days (range, 332–1372). Survival was calculated with the Kaplan-Meier method and survival curves were compared with a generalized Wilcoxon test. Multivariate analysis using Cox's proportional hazards model identified the independent prognostic factors.

RESULTS

Patient Characteristics and Treatments Given

The mean age of the patient was 61.4 years (range, 33–88), with a male-to-female ratio of 3:2. Total gastrectomy was performed in 33 patients, proximal gastrectomy in 4, and distal subtotal gastrectomy in 54. Standard D1 lymphadenectomy was performed in 11 patients (12%), whereas the rest were treated by extended lymphadenectomy (D2 for 41 and D3 or more for 39 patients). D1 lymphadenectomy means systemic resection of perigastric lymph nodes and is a standard procedure worldwide. D2, a standard procedure in Japan, denotes extended lymphadenectomy with resection also of the nodes at the base of the left gastric artery and along the common hepatic and splenic arteries. D3 is an extended procedure with resection of nodes around the hepatoduodenal ligament, behind the pancreatic head, and at the base of the superior mesenteric vein besides performing D2 lymphadenectomy. Adjuvant postoperative chemotherapy was performed in 24 patients, including 5 with positive cytology, but the prognostic impact of the chemotherapy was not significant.

Free cancer cells were detected in 10 patients (11%) overall and in 6 (21%) of 28 patients with microscopically confirmed serosal involvement (pT3 and pT4). Interestingly, free cancer cells were not detected in any of the 4 patients with direct invasion to the adjacent structures (pT4). Correlations between the result of cytologic examination and various clinicopathological factors are summarized in Table I. The incidence of positive cytology results increased significantly as the stage or the pN category (the number of metastatic nodes) progressed. The size of tumor with positive cytology was significantly larger than the tumor with negative cytology ($P < 0.0001$). There appeared to be no correlation between the pathologic type and the positivity of cytology.

Survival of the Patients

Of the 91 patients involved, 18 died of recurrent disease. Survival curves of the patients stratified according

TABLE I. Correlation Between the Result of Cytology Examination and Various Histopathological Factors in Patients Undergoing Curative Resection

	Cytology negative	Cytology positive	*P*
No. of patients	81	10	
Tumor size, cm	62.8 ± 29.7	110.0 ± 51.0	<0.0001
Depth of invasion[a]			
pT2	59	4	
pT3	18	6	
pT4	4	0	
No. of metastatic nodes[b]			0.0005
pN0	22	1	
pN1	37	2	
pN2	19	3	
pN3	3	4	
Clinical stage			0.0179
Stage I	19	1	
Stage II	30	2	
Stage III	26	3	
Stave IV	6	4	
Histopathology			NS[c]
Differentiated	29	1	
Undifferentiated	52	9	

[a]pT2, depth of invasion reaches muscularis propria; pT3, lesion with serosal invasion; pT4, lesion with invasion to the adjacent viscera.
[b]pN0, no lymph node metastasis; pN1, metastases to 1–6 regional lymph nodes; pN2, metastases to 7–15 regional lymph nodes; pN3, metastasis to >15 regional lymph nodes.
[c]Not significant.

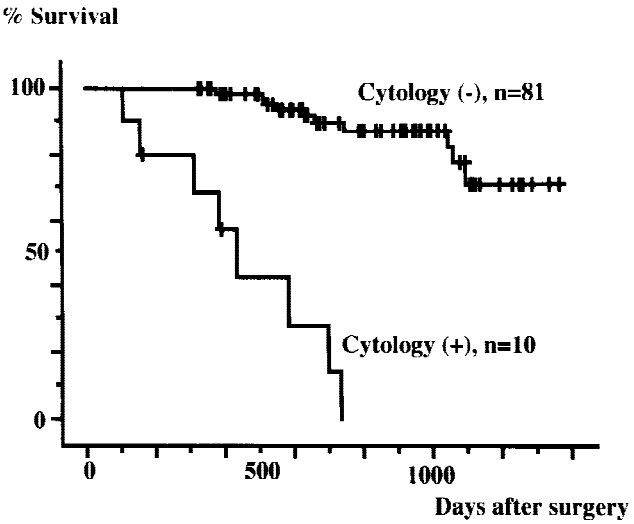

Fig. 1. The survival curves for 91 patients with advanced gastric carcinoma treated by potentially curative resection. The patients were stratified according to the result of the cytologic examination. The patients with a negative cytologic examination had a significantly better prognosis ($P < 0.0001$).

to the results of cytology are shown in Figure 1. The difference between the curves was significant. All patients with positive cytology have died (median time to death, 386 days; range, 109–736 days), except for 1 patient who has palpable recurrent tumor in the Douglas pouch and is currently undergoing chemotherapy 390 days postoperatively. Univariate analysis identified positive cytology ($P < 0.0001$), tumor size >10 cm ($P = 0.001$), and total gastrectomy ($P = 0.0078$) as significant prognostic factors (Table II). Multivariate analysis with these three factors as variables identified positive cytology as the only independent prognostic factor of significance, with the relative risk of 20.4 (Table III).

TABLE II. Univariate Analysis with Various Prognostic Factors as Variables

Variable	Relative risk (95% confidence interval)	*P*
Cytology, positive	21.3 (7.30–63.3)	<0.0001
Tumor size, >10 cm	4.33 (1.61–11.6)	0.0036
Surgical procedure, total gastrectomy	3.67 (1.41–9.54)	0.0078
Serosal invasion, positive	2.08 (0.82–5.29)	0.1219
Node metastasis, positive	3.12 (0.71–13.6)	0.1322
Gender, male	1.31 (0.49–3.49)	0.5953
Location, upper 1/3	1.30 (0.45–3.49)	0.5978
Histology, undifferentiated	1.18 (0.41–3.35)	0.7604

TABLE III. Multivariate Analysis with the Three Prognostic Factors That Were Significant by the Univariate Analysis

Variable	Relative risk (95% confidence interval)	*P*
Cytology, positive	20.4 (5.29–77.9)	<0.0001
Surgical procedure, total gastrectomy	2.62 (0.84–8.17)	0.0975
Tumor size, >10 cm	0.62 (0.15–2.68)	0.5253

Correlation of Cytology Results with Peritoneal Carcinomatosis

Cancer recurrence was detected in 26 patients. Of these, 10 had peritoneal dissemination. Other sites of metastasis detected by various diagnostic procedures included distant nodes in 5 patients, liver in 4, bone in 3, locoregional in 2, and others in 4. Of the 10 patients with a positive cytology result, peritoneal dissemination was diagnosed in 8 patients. The median time to detection of the recurrences in these 8 patients was 222 days after surgery (range, 109–370). Of the remaining 2 patients, 1 died of cardiac failure 164 days postoperatively, and another died of multiple bone metastasis 439 days postoperatively; neither patient showed any signs of peritoneal dissemination at the time of death. Of the 81 patients with negative cytology results, 2 were detected to have peritoneal carcinomatosis 417 days and 546 days postoperatively. The curves for the patients who had positive and negative cytology were plotted by the method of Kaplan and Meier, with a clinical diagnosis of peritoneal dissemination as the end point (Fig. 2). The difference between the curves was significant ($P < 0.0001$).

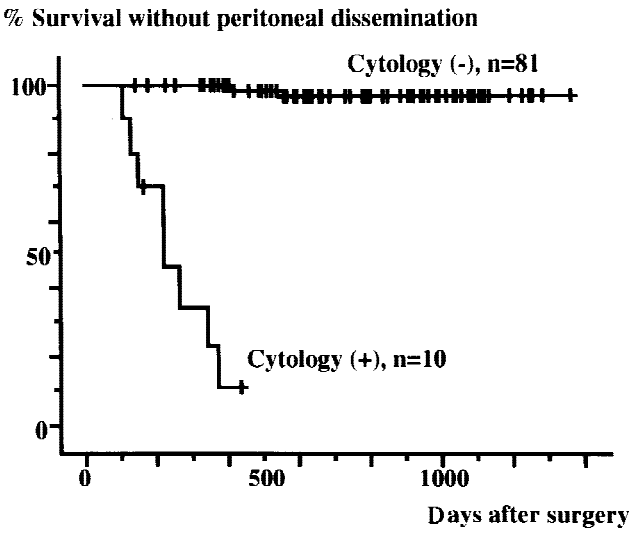

Fig. 2. Curves for 91 patients with advanced gastric carcinoma treated by potentially curative resection, drawn by the method of Kaplan and Meier, with the diagnosis of peritoneal dissemination as the end point. The patients were stratified according to the result of the cytologic examination. Patients with a positive cytologic examination had a significantly increased risk for peritoneal dissemination ($P < 0.0001$).

DISCUSSION

Even if treated by potentially curative surgery, a considerable proportion of patients with gastric carcinoma will die of recurrent disease, with peritoneal dissemination being the most frequent pattern of failure. Some of these patients are known to have free cancer cells in the peritoneal cavity [3], and detection of these cells by cytology is a useful means of identifing a group of patients who are at a high risk of cancer recurrence. Several studies regarding cytologic examination in the past have been performed only for patients with serosal involvement [5,9,10]. However, detection of free cancer cells had been reported in a small number of patients with pT2 tumor [11]. There are two potential explanations for the presence of free cancer cells even without microscopic evidence of serosal involvement. One is that the histological examination of the depth of invasion may not always be performed at a spot where the cancer infiltration is most profound. This could occur from time to time unless several slices per lesion are examined routinely, because the location of the tumor to be sliced for the examination is usually decided macroscopically. Another explanation may be that the cancer cells could be shed through the lymphatics, from the metastatic lymph nodes or through the lymphatic canals via the omentum. Therefore, patients with curatively resected pT2 tumor were included in this study. Consequently, positive cytology results were obtained in 4 patients (6.3%) among this group. Nevertheless, the likelihood of detecting free cancer cells increased as the tumor invasion reached serosa.

The incidence of cytology-positive cases seems to vary considerably among institutions. In a large-scale study performed in conjunction with a Dutch randomized trial comparing D1 and D2 lymphadenectomies, free cancer cells were demonstrated in only 12% of a group of serosa-involved gastric carcinoma patients, who included noncuratively resected cases [6]. Other studies claim that free cancer cells were detected in 18% [9], 18.4% [5], 19.4% [10], 15.7% (pT3) to 34.8% (pT4) [2], and 24% [11,12] of curatively resected serosa-involved cases. In the current study, cytology was positive in 21% of the same group of patients, which is comparable, if not superior, to the result of the aforementioned studies. This sensitivity was found to be sufficient to rank the detection of free cancer cells as the only significant independent prognostic factor in this study. This result clearly justifies the recent revision made in the Japanese staging system for gastric cancer [7], in which a patient with a positive cytology result (termed Cy1) will be classified as stage IV regardless of the extent of other prognostic factors. Cytology in this study identified 1 stage Ib, 2 stage II, and 3 stage III patients who were Cy1 and should be reclassified as stage IV. None of these patients survived. A report from another institution with a lower rate of detection, however, claims that the role of this procedure as a prognostic factor is limited [9] due to false negatives. Cytology with conventional Papanicolaou and Giemsa staining does seem to have a limit, in that it requires expertise from the technicians and pathologists who prepare and examine the specimens.

Despite a reasonably high positivity of the cytologic examination in the current series, 2 (2.5%) out of 81 patients (2.5%) with a negative cytology result were diagnosed later to have developed peritoneal carcinomatosis, although after a longer time interval than the 8 recurrences observed in the cytology-positive group. Several efforts are now underway to raise the sensitivity of the cytologic examination and to decrease the incidence of false-negative results. Peritoneal brushing (sweeping the serosa overlying the tumor with a brush) and imprint cytology (slides coated with poly-L-lysine pressed against the serosa of a resected specimen) are reported to have raised the incidence of detection from 18% (16/85 cases) to 26% (22/85) in a group of gastric carcinoma patients undergoing elective surgery [13]. Wu et al. [10] reported a modified type of peritoneal washing performed by directly rinsing the tumor surface with saline instilled from a height of 20 cm. These methods might raise the sensitivity of the cytologic examination but could induce exfoliation of tumor cells that may not have occurred otherwise. Theoretically, tumor cells that are shed from the involved serosa will have to be viable long enough for several processes needed for the establishment of metastasis to take place. Such viable cells may be included in the specimens obtained by gently rinsing

the cavities far away from the primary tumor (Douglas pouch in particular) but perhaps not among the free cells that were mechanically scraped off from the serosa.

Immunostaining provides another possibility of raising the sensitivity of cytology. For this purpose, however, a panel of several antibodies is usually needed [12,14], because the expression profile of potential target glycoproteins for gastric carcinoma is heterogeneous. Although a rise in detection rate from 24% to 39% for serosa-positive cancer has been reported [12], this process of detection is time-consuming. The use of antibody Ber-Ep4 has circumvented this problem, and a detection rate of 34.2% was achieved for the similar group of patients by the use of this antibody alone [15]. Since Ber-Ep4 staining is frequently observed in macrophages, visible morphology of the stained cells is mandatory to avoid false positives [15]. We are currently attempting another approach, which employs reverse-transcriptase polymerase chain reaction to detect carcinoembryonic antigen mRNA [1]. The current problem with this method is the inevitable emergence of false positives, as well as the greater time and cost required to run the system.

Subset analysis of a recent randomized trial has proved perioperative intraperitoneal chemotherapy to be beneficial in terms of survival for patients with stage III gastric carcinoma [16]. A more aggressive approach with intraoperative chemohyperthermia is reported to be an effective prophylaxis for peritoneal dissemination [17]. Since these treatments generally result in increased morbidity [16], detection of free cancer cells in the abdominal cavity will be useful in identifying patients who are more likely to benefit from these therapeutic options. On the other hand, some investigators who remain skeptical of the effects of these strategies may diagnose their patients as incurable in the event of positive cytology results and decide to perform palliative resection and avoid extended lymphadenectomy for this group of patients [10]. In either case, it is mandatory that the result of cytology be available not long after the beginning of the operation if the knowledge is to be reflected in the treatment strategy. Although several methods are currently available to improve on the conventional method in terms of sensitivity, no technique seems to surpass the conventional one in terms of time needed to obtain the result, which takes <30 min [10].

To conclude, we postulate that peritoneal lavage cytology should be employed for all advanced cancer undergoing potentially curative resection for added accuracy in the stage classification. The results should also be reflected to decide the eligibility of the patients for future clinical trials regarding perioperative intraperitoneal chemotherapy.

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COMMENTARY

This article by Kodera et al. is an excellent example of the significant contribution by Japanese surgeons to the staging of gastric cancer. Previous efforts by Japanese clinicians to precisely define the nodal spread of gastric cancer led to the recent modification of the Tumor Node Metastases (TNM) staging system for gastric cancer to include the number and the location of nodal disease. The Japanese staging system for gastric cancer was also revised not long ago to include peritoneal cytology. Until recently, however, peritoneal cytology for gastric cancer has received little attention in Western institutions. This

article provides further evidence to suggest that peritoneal cytology should be a component of the TNM staging system for gastric cancer. Since the majority of patients with gastric cancer in this country present with advanced disease, accurate pretherapeutic staging is essential to reliably evaluate neoadjuvant chemotherapy trials for gastric cancer. Furthermore, laparoscopically obtained peritoneal cytology may identify a subgroup of patients who are candidates for trials evaluating intraperitoneal chemotherapy or combined routes of administration of chemotherapy (i.e., intravenous and intraperitoneal). Further technical advances, such as immunohistochemistry and polymerase chain reaction (PCR), to increase the yield of tumor cells from peritoneal washings are currently being evaluated to determine the incidence of false-positives and the clinical significance of true-positives.

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